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Evaluation of column bleed by using an ultraviolet and a charged aerosol detector coupled to a high-temperature liquid chromatographic system

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Abstract

In this study, five different HPLC columns were heated to 200 °C using a homemade heating system which can be operated in temperature programmed mode. The column bleed as an indicator of induced degradation of the stationary phase material was evaluated using a charged aerosol detector (CAD) and an ultraviolet diode array detector (UV-DAD) at different wavelengths. The silica based C-18 stationary phase gave the highest bleed, and the carbon clad titanium dioxide column the lowest bleed. This was independent of both the detection technique and the wavelength.

Keywords: Charged aerosol detection; Ultraviolet detection; Stationary phase stability; Temperature programming; HPLC; High-temperature HPLC

1. Introduction

Although the benefits of using high temperatures in HPLC have been reported and reviewed recently [1–4], a major disadvantage for the routine application of high-temperature liquid chromatography is the lack of temperature resistant stationary phases available. Nevertheless, column technology has improved significantly over the last few years and there have been some encouraging reports and reviews concerning extraordinary stable HPLC columns [5–8]. If the column is heated to temperatures up to 200 °C, hydrolysis of bonded phase, dissolution of the support material or column hardware degradation may occur, which is summarized as column bleed. Therefore, a simple, reliable and universal detection technique should be employed to monitor the effluent if the HPLC column is heated to extreme temperatures. This is very helpful for screening experiments, especially if modified stationary phases are tested prior to their routine use for high-temperature liquid chromatography and if MS detection is employed. For detecting particulate matter due to bonded-phase loss in a column under harsh conditions, ultraviolet detection is not always suitable, because the signal depends on the analytes' molar light absorptivity. Charged aerosol detection is a relatively new detection technique based on the previously termed aerosol charging detection by Dixon and Peterson [9], where the eluent is nebulized with nitrogen and the droplets are dried to remove the mobile phase, producing non-volatile analyte particles. A secondary stream of nitrogen becomes positively charged as it passes a high-voltage, platinum corona wire. This charge transfers to the opposing stream of analyte particles and is then transferred to a collector where it is measured by a highly sensitive electrometer. The signal intensity generated by a charged aerosol detector (CAD) is said to be directly proportional to the analyte concentration [10]. This means that the detector response for the bleed of a given column should be correlated to the amount of particulate matter per unit of time. In contrast to this, the UV signal is dependent on the molar extinction coefficient ε. This means that compounds containing weak chromophores give a lower signal than those with a strong chromophoric system if UV detection is used. Therefore, the experiments conducted in this study should evaluate the usefulness of UV and CA detection for the on-line monitoring of HPLC column bleed.

2. Experimental

2.1. Chemicals

Acetonitrile (HPLC gradient grade) was purchased from Baker (Deventer, Holland). High-purity deionized water was

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produced in house by an Elix 10—Milli-Q Plus water purification system (Millipore, Eschborn, Germany).

2.2. Apparatus

All experiments were carried out using a Shimadzu HPLC system consisting of two LC-10AD$_{VP}$ pumps, a DGU-14 A degasser, a SIL 10AD$_{VP}$ autosampler, an SPD-M10A$_{VP}$ diode array detector (Shimadzu, Duisburg, Germany) and a charged aerosol detector (ERC, GmbH, Riemerling, Germany). The CAD was installed directly behind the UV-DAD. The system was controlled via an SCL-10AD$_{VP}$ controller. For data acquisition and analysis the Shimadzu Class VP software (version 6.12 SP3) was used. Furthermore, a homemade heating system was used for controlling eluent and column temperature, which can be operated in temperature programmed mode. Pure deionized water was used as mobile phase. To keep the water in the liquid state a 500 psi back pressure regulator (GammaAnalysenTechnik, Bremerhaven, Germany) was used and connected behind the UVD.

The heating system used in this study was developed for high-temperature liquid chromatography and consists of three modules, all of which can be independently controlled. The heating range of this system extends from room temperature to 225 °C with maximum heating rates of 40 °C/min. The system can be used for isothermal and temperature programmed operations. The first module is the eluent preheating unit containing the stainless steel capillary. The second module is the column heating unit containing the HPLC column. The third module is for eluent cooling and is similar to the preheating unit. The stainless steel capillary and the column are tightly enclosed between two tailor made metal shells. In contrast to commercial heating systems, also the column's end fittings are enclosed to optimize heat transfer. Moreover, this heating system allows for a precise temperature control of all three modules described above, which means that the eluent entering the column and the column itself can be controlled independently. In a previous submitted paper [11], we reported about measuring the eluent temperature in a stainless steel capillary with a very thin thermocouple. We were able to show that even at a flow rate of 2 mL/min and a block temperature of 190 °C, the temperature difference between the heating block and the eluent temperature in a 13 cm long capillary was below 1 °C. To guarantee that the mobile phase entering the column is in full thermal equilibrium with the stationary phase, the length of the heated capillary was increased to 27 cm. Furthermore, the temperature of the preheating module was always adjusted 2 °C above the temperature of the stationary phase. The eluent preheating and column heating unit are equipped with two integrated temperature sensors. The first one is for temperature regulation, the other for temperature recording and verification.

Rapid cooling of the preheating and column unit is achieved by tap water from the laboratory. Behind the block for eluent preheating and column heating is a copper tube where the water is flushed through. After a temperature programme is finished the water cooling is started automatically.

2.3. Dependence of detector response on temperature gradients

The first experiment should evaluate the detector response dependence on eluent temperature for both ultraviolet and charged aerosol detection. A stainless steel capillary was placed in the system instead of a column to generate a blank measurement. In subsequent experiments, five different HPLC columns were then placed in the system and the baseline signal was monitored. It must be noted that the stainless steel capillary for blank measurement was replaced by the HPLC columns and that no further changes to the overall system have been made. Prior to signal recording, each column was flushed with pure acetonitrile at 30 °C to remove strong binding contaminants and was then re-equilibrated with deionized water, which was used as mobile phase. For all experiments, identical conditions concerning temperature programming were maintained. The temperature programme consisted of an isothermal hold up step for 5 min at 30 °C. After this, temperature was raised within 5 min from 30 to 200 °C and was then held constant for 10 min. The system was then cooled down to 30 °C and the baseline signal was monitored for another 20 min. The UV signal was monitored from 190 to 370 nm, although chromatograms shown in this paper are given at a wavelength of 190 and 254 nm, respectively. The eluent cooling temperature was kept constant at 30 °C to avoid any damage to the detector. While the UV detection cell is temperature resistant to at least 80 °C, eluent temperature should not be higher than 60 °C for the charged aerosol detector.

2.4. Description of investigated HPLC columns

HPLC columns used in this study were from Phenomenex (Luna 5 μm C-18, 150 mm × 4.6 mm, Part No. 00F-4041-E0, Serial No. 113499-5), ZirChrom (CARB, 3 μm, 150 mm × 4.6 mm, Lot No. 36-147, Serial No. CARB110901C), Thermo (Hypercarb, 5 μm, 100 mm × 2.1 mm, Column No. 0945316W, Part No. 35005-102146), Polymer Laboratories (PLRP-S 100 A, 3 μm, 150 mm × 2.1 mm, Serial No. 3M-RPS1-124B-90), and ZirChrom-Sachtleben (Prototype-CARB-TiO_2, 5 μm, 150 mm × 2.1 mm, Lot No. 32-143, Serial No. CARBTI011105M). All but the last column are commercially available. Due to a high back-pressure, the gradient starting temperature was set at 90 °C for the PLRP-S column. Otherwise, the upper pressure limit recommended by the manufacturer would have been exceeded. The Luna column is a silica-based C-18 stationary phase. This column, although not specially designed for high-temperature applications, was chosen due to the high popularity of silica-based materials and to compare the bleed of this column to columns which exhibit good or excellent pH stability. The Hypercarb column consists of pure graphitized carbon and is said to be stable up to 200 °C and over the whole pH range. The ZirChrom-Carb column consists of zirconium dioxide as support material. The surface has been cladded with carbon and therefore, this column should be very stable at extreme pH and high temperatures, although the temperature limit is set at 150 °C by the column manufacturer. The carbon clad titanium dioxide column is similar to the ZirChrom-Carb column, with the dif-